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ABSTRACT

Research in human genetics is now expanding rapidly, leading to increasingly precise ways of preventing or treating some of the 2,000 or more inherited disorders that afflict human beings. At the same time, it has produced a wealth of new ideas and techniques which are laying the groundwork for new medical science for the 21st century. Recent work with man-mouse hybrid cells, for instance, is making it possible to map which human genes belong on which chromosome; the hybrid cells are also allowing researchers to investigate how individual genes can be "turned on" and how the body reacts to viral infection. Much of this progress is due to scientists' new ability to grow and study live human cells. Hundreds of experiments which could not be done on human beings can now be carried out on cells grown from small slivers of skin or other tissue taken from people with genetic disorders. This pamphlet describes how the National Institute of General Medical Sciences' new library of human cell lines (the Human Mutant Cell Repository in Camden, N.J.) grows, stores and distributes such cells, and how they are used to help geneticists in their research. (Author)

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THE NEW HUMAN GENETICS

A Cell Bank Helps Researchers Fight Inherited Disease

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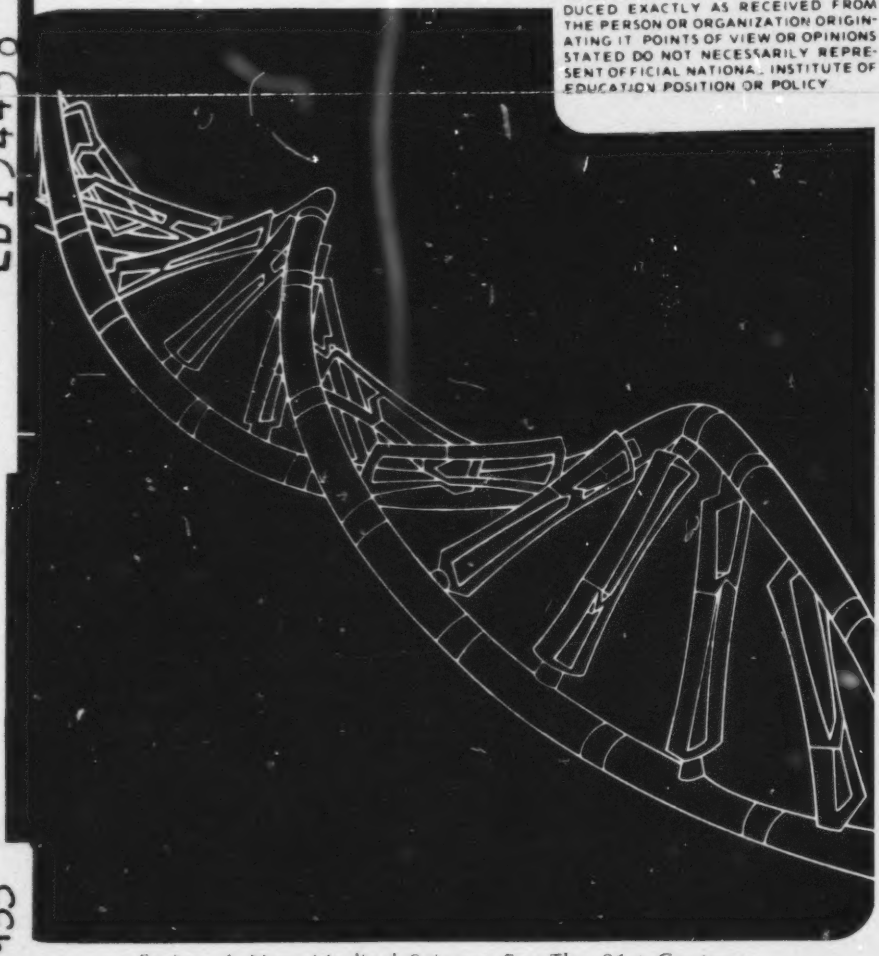

Series: A New Medical Science For The 21st Century

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What is NIGMS?

The National Institute of General Medical Sciences (NIGMS) is unique among the National Institutes of Health in that its main mission is the advancement of the basic medical sciences. It supports selected research and research training programs in areas that underlie all medical investigation; namely, cell and molecular biology and genetics. Ten of the scientists whom it supported have won Nobel Prizes since 1968 for their pioneering work on the structure and function of genetic material, the action of hormones, and the structure of the cell. Knowledge resulting from this work contributes directly to the progress of research in the other institutes of NIH. The Institute also develops and supports interdisciplinary studies in pharmacology, toxicology, anesthesiology, biomedical engineering, epidemiology, and trauma and burn research.

THE NEW HUMAN GENETICS

A Cell Bank Helps Researchers Fight Inherited Disease

by Maya Pines

Series: A New Medical Science for the 21st Century
The National Institute of General Medical Sciences (NIGMS)
DHEW Publication No. (N.I.H.) 76-662

"The cost of medical care is at its highest, and the technology at its most difficult, during the time when we must work with an incomplete understanding of disease. Whenever we have gained a genuine, penetrating insight, based on hard facts, and then developed a direct, decisive technology on the basis of this, it has always turned out to be relatively simple and relatively cheap. . . . My advice is to go for the fundamental kinds of information, for that is where the great pay-offs will be." **Lewis Thomas, President, Memorial Sloan Kettering Cancer Center.**

SUMMARY

Research in human genetics is now expanding rapidly, leading to increasingly precise ways of preventing or treating some of the 2,000 or more inherited disorders that afflict human beings. At the same time, it has produced a wealth of new ideas and techniques which are laying the groundwork for a new medical science for the 21st century. Recent work with man-mouse hybrid cells, for instance, is making it possible to map which human genes belong on which chromosome; the hybrid cells are also allowing researchers to investigate how individual genes can be "turned on," and how the body reacts to viral infection. Such work is relevant not only for the many diseases that have clear-cut hereditary causes, but for countless other disorders in which genetic factors play a less defined but important role.

Much of this progress is due to scientists' new ability to grow and study live human cells. Hundreds of experiments which could not be done on human beings can now be carried out on cells grown from small slivers of skin or other tissue taken from people with genetic disorders. This pamphlet describes how the National Institute of General Medical Sciences' new library of human cell lines (the Human Mutant Cell Repository in Camden, N.J.) grows, stores and distributes such cells, and how they are used to help geneticists in their research.

Saved by specialists in human genetics, he is now doing fine.

THE NEW HUMAN GENETICS:
A Cell Bank Helps Researchers Fight Inherited Disease

An 8-months-old boy was brought into the hospital, in a coma and near death. While doctors gave him emergency treatment, researchers tried to find the cause of his sudden illness. Examining samples of his urine, they discovered large quantities of methylmalonic acid, which is usually present to this extent only in cases of vitamin B-12 deficiency—yet his blood showed plenty of vitamin B-12.

Specialists in the new science of human genetics were called in. They took a small sliver of skin from the boy's forearm, minced it and put it in a culture medium where the cells could grow and divide every 18 hours. Then they studied how these cells responded to various chemicals.

Their tests pointed to an unusual defect: The boy's cells lacked a certain enzyme which normally activates vitamin B-12 and allows it to break down the methylmalonic acid into simpler chemicals. Because of this genetic defect, he needed 1,000 times the normal amount of vitamin B-12; no lesser amount would do. The doctors then injected an enormous dose of the vitamin—and as if by miracle, the boy recovered. The quantity of methylmalonic acid in his urine decreased dramatically. The basic defect remained, but the doctors expected that with continuous high doses of vitamin B-12 he would develop normally. Their prediction came true. Even though he was quite retarded in physical and mental development before his treatment began, by the time he entered the third grade he had caught up in nearly all respects.

Not only did the scientists save the boy's life in this way, but they identified a new type of inherited metabolic disease. This had three further consequences, explains Dr. Leon Rosenberg, director of the Genetics Center set up by the National Institute of General Medical Sciences at Yale University Medical School, where these events took place in 1968:

(1) Having grown human skin cells (fibroblasts) from this particular boy and compared them to cells from normal human volunteers, the researchers were in a position to diagnose this defect in other patients efficiently and rapidly. They have now done so in dozens of cases, working with cells supplied by medical centers in the U.S. and abroad.

(2) Stimulated by this first documented example of a genetic disorder involving vitamin metabolism, other researchers have discovered some 25 similar illnesses, many of which affect the central nervous system and produce mental retardation, seizures, gait abnormalities or behavioral disorders resembling schizophrenia.

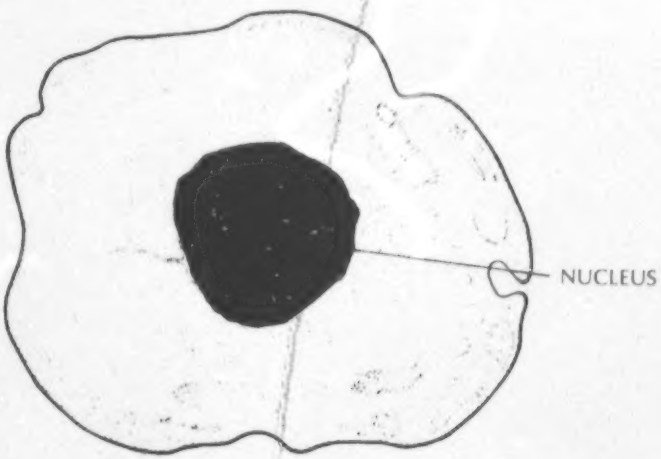
(3) Using cells cultured from amniotic fluid, the Yale scientists learned to detect the disorder *prenatally*. When the mother of the little boy who nearly died from this defect became pregnant again, for instance, they were able to reassure her early in her pregnancy that her next child would be all right. So was her third child. However a woman in Stockholm, Sweden, whose first child had died from the disease a few weeks after birth, was found to be carrying a fetus that was also affected. She elected to have an abortion. When she became pregnant a third time, the doctors were able to report the good news that the fetus was spared, and she gave birth to a healthy child.

"To my own view, the most startling development of the decade, and the one most replete both with opportunities and with policy dilemmas, has been the rapid development of fetal diagnosis . . . the detection of hereditary disease in utero. . . ." **Joshua Lederberg, Prof. of Genetics, Stanford University.**

The Beginning of Human Genetics

Human genetics research is now advancing so rapidly and on so many fronts that it seems strange to realize that only two decades ago—when Dr. Rosenberg went to medical school in 1954, for instance—human genetics was not even a part of the curriculum. In fact, there was no such field. Only a few people had succeeded in growing human cells outside the body. Until 1956, scientists didn't even know the correct number of chromosomes, the rod-shaped bodies of genetic material (genes), in a human cell.

They had learned a great deal about bacteria, which they knew how to grow on a large scale in a culture medium. Since bacteria reproduce about once an hour, millions of their progeny could be obtained in a very short time. Bacterial cells are also very much simpler than mammalian cells, and far easier to study. (For example, their chromosomes are naked within the cell, rather than joined with proteins and encapsulated



A human cell: The nucleus contains the genetic material, but the chromosomes (rod-shaped bodies bearing the genes) become visible only during cell division.

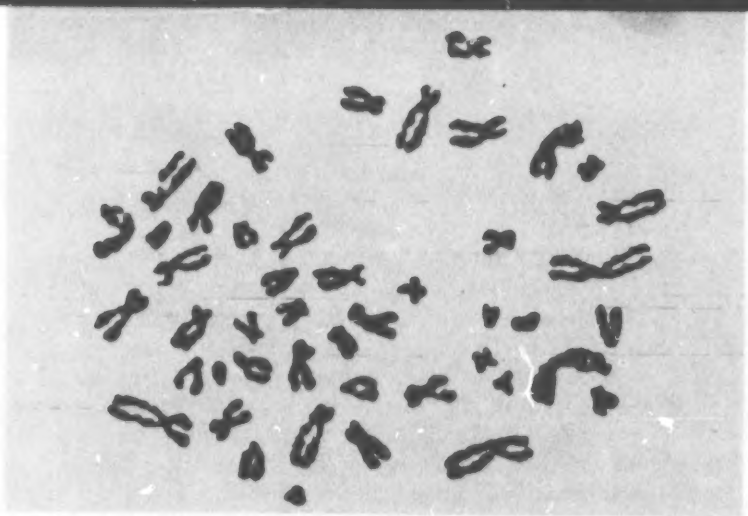
inside a nucleus like those of mammalian cells.) But mammalian genetics still depended on the slow-paced method of mating two animals and observing their offspring.

This worked to some extent with mice. Since human beings cannot be subjected to controlled breeding experiments and furthermore have relatively few children, who take a long time to grow up and reproduce, however, the study of human genetics was particularly difficult. It remained largely an *observational* science, much like atomic physics between the time of the Greeks and the 19th century. As earlier physicists had deduced the existence of atoms from the properties of matter, so geneticists deduced the existence of genes from the properties of organisms and their progeny; but they could neither manipulate nor transform the particles about which they built elaborate theories. Their experiments were at the level of the total organism, rather than of the chromosome or the gene.

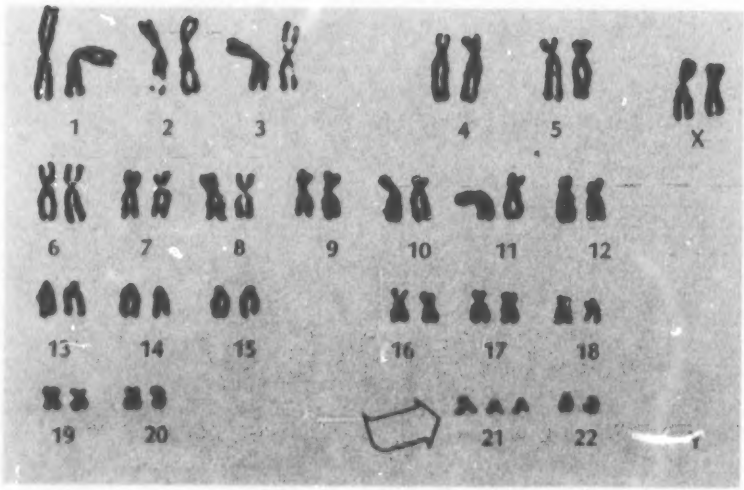
Today human genetics is at the forefront of a scientific explosion which is changing our whole approach to medical research. Far from being limited to observation, human genetics has pioneered in the development of subtle and precise methods of fighting disease that make use of the delicate machinery inside the human cell.

A New Understanding of Chromosomes

The new human genetics really started with Joe Hin Tjio and Albert Levan's demonstration, in 1956, that there are 46 chromosomes inside each human cell. This achievement was made possible by several advances in the technology of human tissue culture, particularly the use of colchicine, a chemical which arrests cells at the very moment, during cell division, when their chromosomes are most easily visible. The chromosomes can then be distinguished from one another and their shapes studied individually. Tijo and Levan's techniques were quickly copied by other workers.



With this historic photo, Drs. Joe Hin Tjio and Albert Levan showed that there are 46 individual chromosomes in a normal cell. (Magnification: × 2,400.) Half of these chromosomes come from the mother, half from the father, and they are usually counted in pairs—except for the sex chromosomes (X and Y chromosomes).



In Down's syndrome (mongolism), there is an extra chromosome No. 21 (three rather than the normal two.) The extra chromosome is believed to result from incomplete separation of the chromosomes during the formation of the egg cells by the ovary. The condition thus represents a genetic accident rather than a strictly heritable disease.

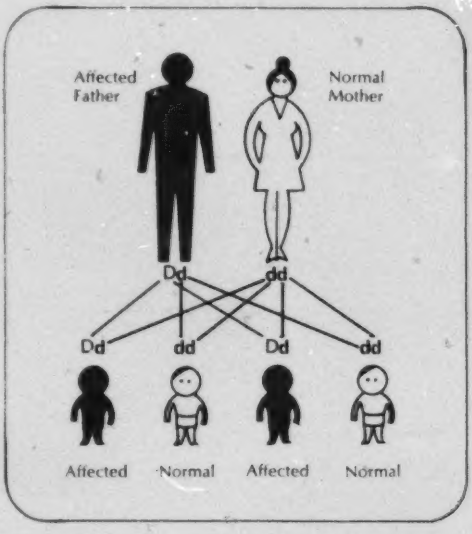
Within three years, researchers had cracked a major mystery: They showed that Down's syndrome (mongolism), which produced mental retardation and characteristic facial features in one out of 600 American children, was caused by a single extra chromosome in each cell. With this clue they were able, for the first time, to detect Down's syndrome before a child was born. By culturing fetal cells obtained from a sample of amniotic fluid and then counting their chromosomes, they could make their diagnosis early enough in pregnancy to give the parents the choice of having an abortion. Thousands of parents have taken advantage of this forecasting ability, especially when the mother was in the age range—over 40—that stood in greatest danger of having a Down's syndrome child.

This discovery led to many others, as Turner's syndrome (short stature, webbed neck, cardiac defects, and infertility in females), Klinefelter's syndrome (long legs, incomplete sexual development in males) and dozens of other disorders were traced to either an extra or a missing chromosome, or to missing pieces and rearrangements of the chromosomes in a cell.

How Dominant Inheritance Works

One affected parent has a single faulty gene (D) which *dominates* its normal counterpart (d).

Each child's chance of inheriting either the D or the d from the affected parent is 50%.



How X-Linked Inheritance Works

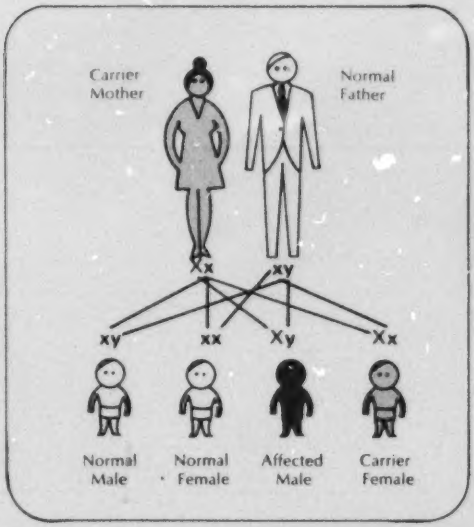
In the most common form, the female sex chromosome of an unaffected mother carries one faulty gene (X) and one normal one (x). The father has normal male x and y chromosome complement.

The odds for each *male* child are 50/50:

1. 50% risk of inheriting the faulty X and the disorder
2. 50% chance of inheriting normal x and y chromosomes.

For each *female* child, the odds are:

1. 50% risk of inheriting one faulty X, to be a carrier like mother
2. 50% chance of inheriting no faulty gene.

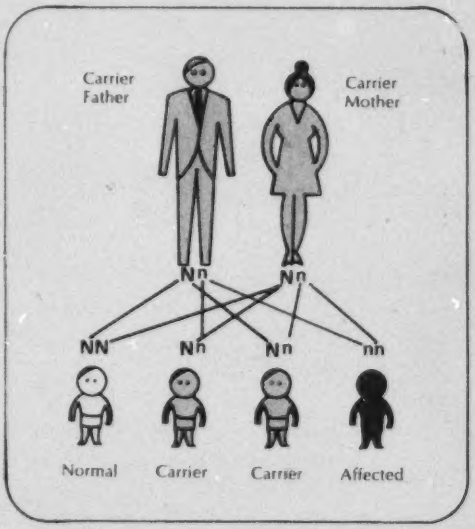


How Recessive Inheritance Works

Both parents, usually unaffected, carry a normal gene (N) which is generally sufficient for normal function despite the presence of its faulty counterpart (n).

The odds for each child are:

1. a 25% risk of inheriting a "double dose" of n genes which may cause a serious birth defect
2. a 25% chance of inheriting two Ns, thus being free of the faulty gene
3. a 50% chance of being a carrier like both parents.



New Ways of Studying Inherited Biochemical Defects

Meanwhile a Japanese otolaryngologist noticed that one of his patients had a very peculiar reaction to hydrogen peroxide: in his mouth, the peroxide never bubbled, as it should, when poured over a wound. The doctor's observation led to the identification of acatalasemia, a recessive trait so rare that no patients could be found with this defect in the U.S. American investigators then obtained samples of cells from the mouths of Japanese patients, and after culturing them, demonstrated that the cells lacked catalase, a specific enzyme which breaks down hydrogen peroxide.

"This opened up the intriguing opportunity to study human genetic disease by methods familiar to the microbiologist, using not bacteria or yeast but cell lines derived from patients exhibiting specific genetic defects," declared Dr. DeWitt Stetten, Jr., former director of NIGMS and now NIH's Deputy Director for Science.

Scientists could suddenly move on from the traditional Mendelian study of how traits are transmitted to "expression" genetics—the study of how genes act biochemically at the level of molecules or cells. And it turned out that diseases which seem, in a patient, to be localized to a particular organ, are very often caused by a biochemical defect which shows up (is "expressed") even in cells taken from his skin.

One of the most exciting developments in genetics in recent years, for example, was the discovery of the basic defect that produces atherosclerosis and heart disease in people with familial dominant hypercholesterolemia. This defect leads to early death from heart attacks associated with extremely high concentrations of cholesterol in the blood. Drs. Joseph Goldstein and Michael Brown of the University of Texas were able to find its mechanism simply by studying cultures of skin cells (fibroblasts) taken from patients with the disease. First they analyzed the sensitive feedback system through which normal human fibroblasts turn off the

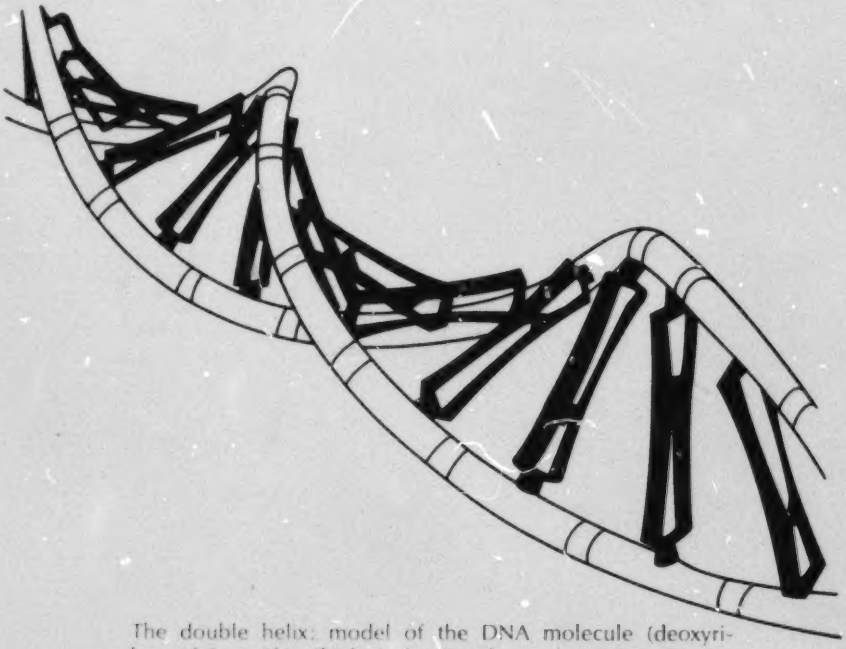
"Almost everyone knows that the science of genetics was founded by a monk named Gregor Mendel, who carried on plant breeding experiments as a hobby in the garden of his monastery in Moravia. . . . The essence of Mendel's discovery was that hereditary traits—or 'characters'—are independent of one another and each is transmitted as a separate unit from a parent to the offspring; in other words, the organism is a mosaic of distinct and independent qualities.

This view contrasts with the older idea that the characteristics of the parents are blended in the offspring, as one might blend two liquids by mixing them together." **Norman H. Horowitz, Prof. of Biology, California Institute of Technology.**

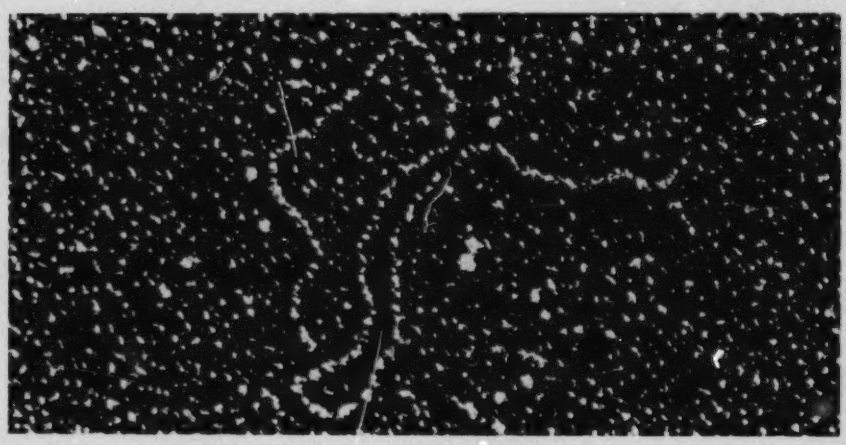
production of cholesterol when they receive information that enough has been manufactured in the body—a system which depends on the effects of a specific lipoprotein in human blood. The cells cultured from patients with familial hypercholesterolemia failed to respond to this crucial lipoprotein, however, and went right on producing cholesterol. Further experiments zeroed in on a defect in these cells' membrane—a lack of certain receptor sites—which broke the normal chain of control.

For the first time, in this way scientists had a clear cell model of a specific disease caused by a dominant gene. They could demonstrate how the lack of a receptor on the cell's membrane interfered with normal feedback from the body, leading to over-production of cholesterol, and they could experiment with different forms of therapy—all in the cell.

They could see that certain chemicals now used to treat familial hypercholesterolemia did temporarily reduce the quantities of cholesterol in the cell, but that these drugs also led the cell to produce even more cholesterol to make up for the loss. This explained why the drugs often had little success. A new drug which they are now trying out in tissue culture may solve the problem, specifically reducing the cell's ability to make



The double helix: model of the DNA molecule (deoxyribonucleic acid,) which embodies the code of heredity, as conceived by Nobel Prize winners Francis Crick and James Watson in 1953. All living things reproduce themselves according to the genetic information in different sequences of DNA sub-units (nucleotides.) Genes, the units of heredity, consist of nucleotides linked together in a helical or spiral form.



A piece of DNA as seen under an electron microscope. It is actually .000055 inches long.

cholesterol rather than the amount of cholesterol that is already made. Drugs developed in this fashion may prove to be widely applicable.

Among people who have suffered heart attacks before the age of sixty, one out of twenty has been found to carry the gene for familial hypercholesterolemia. The gene actually occurs in roughly one in 500 persons in the general population; it is thus one of the most prevalent genes which, in single dose, lead to a specific and sometimes fatal disease. Should two persons with this gene marry and transmit a double dose of it to their offspring, the latter stand a high chance of dying from heart disease before the age of 25. Even a single dose will result in a doubling or tripling of normal serum cholesterol levels from the time of birth. An effective treatment for this disorder might help hundreds of thousands of Americans who carry this gene. Millions more who suffer from similar defects, perhaps caused by the interaction of several genes, might also be helped.

Genetic Disorders: Widespread, Varied, Increasingly Recognized

One by one, other medical mysteries are also yielding to the genetic approach, and an ever-growing number of diseases have been found to be inherited. Not too long ago, genetic diseases were considered quite rare. But today it is recognized that innumerable persons suffer the consequences of disorders due wholly or in part to a defective gene or chromosome. Some 2,000 different genetic disorders have been identified. They make up a very diverse group, including:

(1) Blood disorders; sickle-cell disease, for instance, affects some 50,000 Americans, mostly blacks, producing chronic anemia, jaundice, severe pains in the abdomen and joints, poor resistance to infection, and sometimes an early death. Over 2 million Americans are carriers of this disorder,

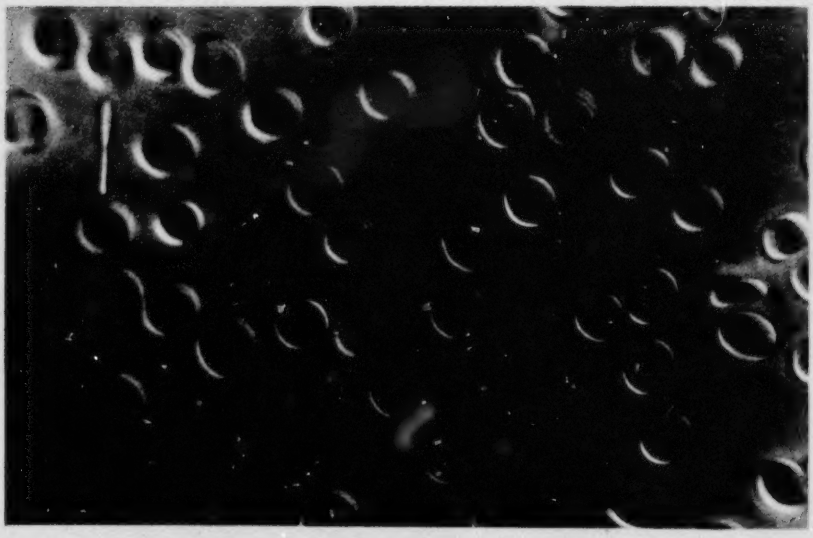
caused by an easily identified recessive defect in a single gene. If they inter-marry, their children run a 25 percent risk of having sickle-cell anemia. Researchers are now studying various techniques for detecting the defect prenatally. Meanwhile, several possible methods of treatment are under investigation.

(2) Abnormal reactions to various foods or drugs. For example, an estimated 100 million persons around the world suffer from an inherited deficiency of the enzyme G6PD. Some of them may contract severe hemolytic anemia if ever exposed to such common drugs as aspirin, the sulfa drugs, vitamin K, certain antimalarials, or even moth balls.

(3) Several forms of mental retardation, such as Down's syndrome and PKU (phenylketonuria), a condition caused by the inability to metabolize the amino acid phenylalanine. Since Down's syndrome results from a chromosomal abnormality, nothing can be done to treat it with present methods. With PKU, however, a diet low in phenylalanine can reduce or prevent brain damage and mental retardation if it is started immediately after birth. Screening for PKU is now mandatory in many states. It is also possible to identify carriers of the trait, though prenatal detection is still beyond reach.

(4) Degenerative diseases that appear in adulthood —for example, the dreaded Huntington's disease. Unlike other genetic disorders that show up shortly after birth, Huntington's disease may not make itself known until the victim is 35 or 40 years old. Then it produces progressive and irrevocable deterioration of the brain. First comes a change of personality, then involuntary movements of the face, hands and shoulders (thus the older name for the disease is Huntington's "Chorea," derived from the Greek word "choros" for "dance".) Facial expressions become dis-

NORMAL CELLS



SICKLE CELLS

torted. Speech blurs. As the disease advances, the victim becomes totally disabled and then irrational. It is a slow process, leading to death within 10 to 15 years. There is no cure. Nor can the disease be detected before birth. Since it is caused by a dominant gene, any child of an afflicted parent stands a 50-50 chance of developing it later in life. Unfortunately, the illness is often diagnosed long after the victim has had children of his own; his offspring must then live in fear of suffering the same fate. At present there are few clues to the mechanism of action of the defective gene.

(5) Behavioral disorders. This is the most poorly defined category of genetic disorders, though clearly there is a strong genetic component to all behavior. Animal breeders have succeeded in breeding special strains of dogs, mice and other animals for gentleness, fierceness, "maze-brightness" and other traits. In human beings, researchers have found evidence of a large genetic component in that most widespread of mental diseases, schizophrenia. Judging from recent studies, manic depressive illness can be transmitted by a gene on the X chromosome. But since behavior cannot be measured as precisely as physical traits, it has been very difficult to classify behavioral disorders according to their biochemical correlates. There has been much discussion, recently, about the effects of an extra male chromosome (XXY) and its implications for antisocial behavior, but little is yet known about them. Compared to other aspects of heredity, the field of behavioral genetics is still in a fairly primitive state.

Researchers still face a formidable challenge with some genetic diseases. Some 10 million Americans are carriers of cystic fibrosis, for instance, a disease which may threaten the life of their offspring if they inter-

marry. Until twenty years ago, a child who was afflicted with it stood little chance of surviving to his 6th birthday. The thick mucus that formed in his pancreas and lungs prevented normal digestion or breathing, and he would either starve despite an adequate diet, or die from severe infections. Even though enzyme therapy and antibiotics now help many of these children survive to adulthood, they still require constant care, including regular drainage of their lungs and other special measures to get rid of the mucus.

Cystic fibrosis is the most widespread recessively inherited disease among white Americans, yet to date its carriers cannot be readily detected. The basic biochemical defect is not known. Nor can the disease be identified prenatally. However, some recent experiments show that certain substances in the blood of patients will make an oyster's gills release a cloud of mucus and affect the motion of the cilia. Researchers are now following up this lead in the hope that accurate methods of detection may be developed in the future.

"To be conservative, let us assume that the human species has only 1,000 genes and that each gene has only two variants. Even on this conservative basis, Mendelian segregation and recombination would be capable of producing 2^{1000} different gene combinations in human beings. The number 2^{1000} is easy to write, but is utterly beyond comprehension. Compared with it, the total number of electrons and protons estimated by physicists to exist in the universe is negligibly small! Natural selection tests the fitness in certain environments not of single genes, but of constellations of genes present in living individuals." **Theodosius Dobzhansky, Prof. of Genetics, University of California—Davis.**

Fortunately in many other genetic diseases the researchers' power of prediction and prevention has grown by leaps and bounds. By now they can diagnose 80 different genetic disorders prenatally, and more keep being added to the list. Genetic counseling has proved increasingly useful. Dozens of new techniques for treating genetic diseases have been developed, involving either a change of diet (as in PKU), the removal of substances that act as toxins in the body (such as copper in persons suffering from Wilson's disease), the administration of genetically required substances (such as vitamin B-12 in the case described on page 5) or organ transplants (for persons with certain inherited immune deficiencies.)

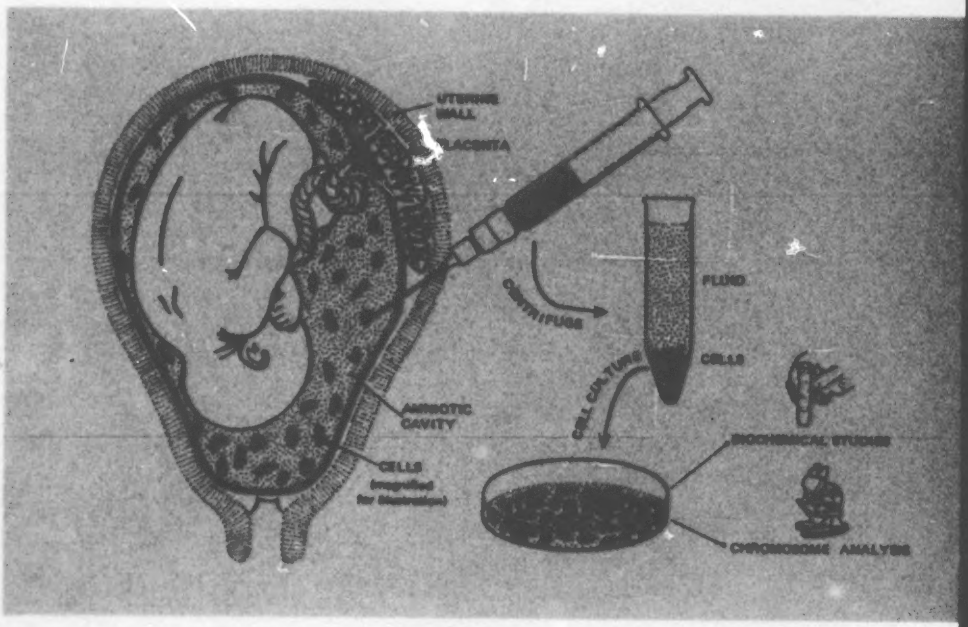
Beyond these dramatic improvements in specific prevention and treatment, research in human genetics has led to a torrent of new ideas and techniques that are on the verge of revolutionizing medicine. This work is relevant not only for the many diseases that have a clear-cut hereditary cause, but for countless other disorders in which genetic factors play a less defined but important role. It has implications for the study of cancer, heart disease, schizophrenia, diabetes, high blood pressure and many other ills. It is throwing new light on how the body reacts to viral infection. It is showing why individuals vary so much in their reaction to drugs. It is tackling such fundamental problems as how people grow, and what happens during aging.

A key factor in the increasing tempo of this research has been the recent progress in methods of culturing human cells—and the greater availability of the cells themselves.

It is no longer necessary for scientists who wish to study certain genetic disorders to spend years trying to develop lines of human cells that express these disorders. Nor need they limit their studies to the few genetic diseases for which they happen to have cells in their laboratories. Instead, they are now free to

approach genetic problems much more imaginatively. They can select exactly the kind of human cells they need, combine and recombine different types to their specification, and attack specific questions with elegance and efficiency.

Very often all they need to do is to apply to the national library of human cells which NIGMS established in 1972 at the Institute for Medical Research in Camden, New Jersey.*



Amniocentesis—A relatively simple and safe method of determining whether a fetus suffers from a suspected genetic abnormality. Cells shed by the growing fetus are cultured from a sample of amniotic fluid, withdrawn from the expectant mother's uterus by means of a hypodermic needle. The cells are then tested for biochemical or chromosomal defects. Some 80 serious genetic diseases, including Down's syndrome, can now be detected prenatally in this way.

* Copewood Street
Camden, New Jersey 08103

A New Library of Human Cells

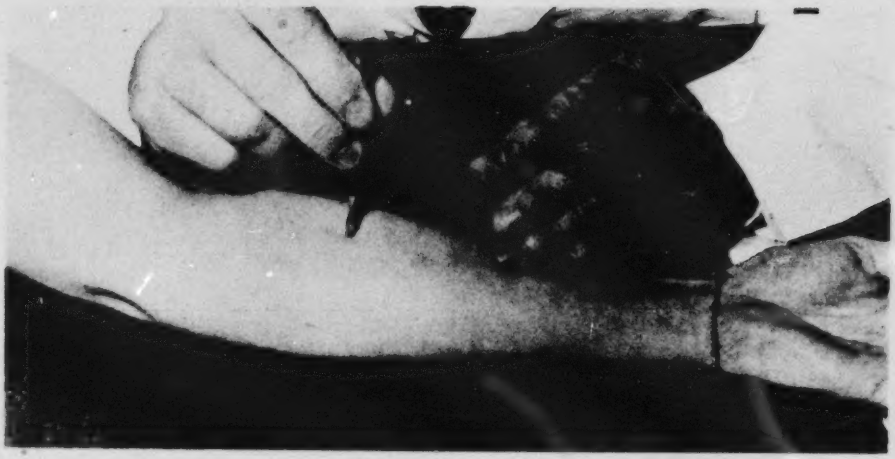
This "Human Mutant Cell Repository" is a unique national resource that will eventually contain cell cultures representing most of the important genetic diseases. At present it grows, stores and distributes cultures of live human skin cells (fibroblasts) originally taken from patients with 49 genetic disorders that are expressed biochemically in these cells; 20 genetic diseases not yet expressed biochemically in fibroblasts, at least with present methods; 48 chromosomal abnormalities; and a dozen other conditions, as well as fibroblasts from unaffected carriers and normal volunteers, to serve as controls. In addition, it has several types of human lymphoid cells (grown from white blood cells) and cells taken from human amniotic fluid. These cells are available at low cost to anyone doing research on the early diagnosis, prevention or treatment of genetic diseases. A number of consultants who are experts in specific genetic diseases help the Repository select new cell lines. In 1974, the Repository distributed over 800 cell cultures to various researchers.

It is a striking characteristic of normal human fibroblasts that they reproduce only a limited number of times in tissue culture—usually not more than 40 or 50 times. Well before the end of this lifespan, they slow down, taking much longer to divide. Researchers therefore need cells that are still in their prime. "Before the Repository, if you wanted to work on a genetic disorder like galactosemia, which is expressed in fibroblasts, you usually got a cell line from a friend," explains Dr. Fred Bergmann, director of the Genetics Program at NIGMS. "But the cells could be contaminated, or half dead! So the Repository does not usually begin with a tissue culture, but with a primary source—a piece of skin from a patient."

A Family Contributes Samples of Its Skin

On a recent day at the Repository, for instance, 30 specimens arrived by air from New Orleans—the result

A piece of skin is removed from a young woman's forearm. After incubation in a nutrient solution, some of the cells in this tissue will grow and divide, to form a line of cultured cells.



of a large family reunion in that city, during which a doctor had taken biopsies from the arm of each person because the family was afflicted with a rare disease, homocystinuria. (A child who inherited the faulty genes from both parents would be mentally retarded and would excrete homocystine in his urine because of a liver defect.) Skin biopsies such as these are usually taken with a local anesthetic. A piece of skin about 3-4 mm. in diameter is cut out, scooping up just enough to go through the superficial skin layer. The piece is then dropped into a narrow specimen tube filled with culture medium (such as McCoy's 5-A medium, which includes essential vitamins, amino acids and minerals, plus calf serum.) Since skin is not usually sterile, an antibiotic such as penicillin or streptomycin is added. The tube is then closed and sent out by air mail.

As soon as a specimen reaches the Repository, it enters a long chain of operations done under scrupulously sterile conditions to turn each specimen into 50 or 100 ampules of noncontaminated live cells representing very specific genetic disorders and guaranteed

to be "at low passage"—meaning that they have divided no more than a limited number of times.

How Human Cells Are Planted and Harvested

First a technician wearing a green surgical smock and a paper cap to cover his hair washes the tissue three times in fresh medium, in three separate Petri dishes. (All the media used at the Repository are themselves carefully checked for sterility before being used.) Then he opens a set of sterile instruments: two scalpel blades and one forceps. With these he cuts up each specimen into tiny pieces, using the scalpel blades delicately in criss-cross fashion, for the specimens must not be torn, or they will not grow properly. Lifting them up with a pipette, he then "plants" the eight or ten pieces in a plastic flask with flat sides, spacing them out carefully along one of the sides so that they take up the whole area without crowding one another. Covered with fresh culture medium and resting on the side of the flask, they are left to incubate at body temperature.

At the end of one or two weeks, the side of the flask is covered with cells. It looks slightly cloudy and opaque. This means that it is time for the first harvest. The cells are loosened from the plastic with trypsin (a pancreatic enzyme) and counted. They are then distributed into two new flasks, each one containing between 100,000 and 500,000 cells. They are allowed to grow another week, then transferred again, producing four flasks at this second "passage." When enough cells have been grown—usually after the third or fourth passage—they are ready to be frozen. The cells are then counted and distributed into small ampules made of special glass that withstands extreme cold, about one million cells to an ampule. The ampules are sealed with an oxygen flame, then slowly cooled, at a rate of one to five degrees per minute, until they are frozen. Finally they are stored in liquid nitrogen at a temperature of 316 degrees below zero Fahrenheit, in gigantic thermos bottles of stainless steel.

A Place Where Even Mops Are Sterilized

Meanwhile there have been regular checks for sterility and activity all along the line. The Human Mutant Cell Repository is a place where even the mops are sterilized. The sterile rooms in which technicians work are washed down several times a day, as soon as the work with any particular cell culture is finished. After the table tops are disinfected and the floor has been mopped with a sterile mop, a vacuum sucks up all the water from the floor to prevent the formation of aerosols. The mops are then sent to the autoclave to be boiled at high heat, rinsed with distilled water, wrapped in parchment paper and autoclaved again to sterilize them. The technicians themselves must keep their arms and hands scrupulously clean; should they happen to touch their nose or mouth, they must stop their work immediately and go wash up again. For fear of contaminating the cultures, they are not allowed to talk in the sterile rooms. Most of the time their work is performed under laminar flow hoods; these bring in streams of filtered air which flows down towards the tables, and then out to an air vent under the tables, so that the technicians neither breathe onto the cultures nor run much risk of being infected by them. The filters make the air essentially sterile against bacteria, viruses, and also mycoplasma, tiny organisms (some of which are called PPLO for "pleuropneumonia-like organisms") which are difficult to identify in cultures but which may produce disease and whose presence can invalidate research results.

While cells from a newly-arrived biopsy are growing and before their first transfer, a few samples are removed for culture in five different media and on blood sugar plates to test them for contamination by bacteria, yeasts, molds and fungi. Other samples are sent to the Repository's special mycoplasma lab for a variety of tests, including radio-isotope tests, that take three weeks. If the cells are found to be contaminated, all flasks of the new line are thrown out to prevent the

At the cell bank, a laminar flow hood protects cell cultures against airborne contamination. A stream of filtered air flows down toward the table at which the technician works, and out to an air vent under the table.

cross-contamination of other cultures. If there is no contamination, they are checked again just before freezing. Even then, another trial awaits them: Some time after they have been stored in liquid nitrogen, a few ampules are retrieved and thawed out for final checking. First they must go through the whole gamut of sterility tests. Then technicians check them to make sure that whatever characteristics originally brought them to the Repository have survived. For instance, if the cells were derived from a child with Down's syndrome, they must still show the characteristic extra chromosome. If derived from a galactosemic, the affected enzyme system must still be defective. This final confirmation of specific defects is carried out either at the Repository or by consultants in medical centers throughout the country. Only after this has been completed is the new line listed in the Repos-

tory's catalogue and made available to researchers. The entire process, from biopsy to frozen ampule and confirmation, takes between three and six months.

Besides the samples of skin from the 30 members of one family in New Orleans, the Repository recently received biopsies and a few cultures of rare fibroblasts from such far-flung places as Helsinki, Toronto, Paris, Winnipeg, Chapel Hill, Los Angeles and The Bronx. A series of biopsies from several families afflicted with familial hypercholesterolemia in Lebanon was sent in by an American doctor from New Jersey who collected them in the Middle East. In addition, Dr. Goldstein of the University of Texas, who discovered the cellular mechanism responsible for this genetic disease, sent in a culture of the cells on which he had based his studies. Word of his contribution got around rapidly, and even before the Repository's new catalogue listing this culture was mailed out, half a dozen requests came in from researchers who wanted to work with this line.

The Need For More Specialized Human Cells

Useful as fibroblasts are, they do not fill all the needs of researchers in the new genetics. One problem is that their lifespan is limited to some 50 generations (although of course this may prove helpful to scientists who are investigating the process of aging.) From the point of view of those who do biochemical studies, an even greater handicap of fibroblasts is that they are among nature's least specialized cells. They do not do any of the specific jobs of liver cells, kidney cells, or any other specialized cells, so that a defect in the body's ability to produce certain enzymes which are manufactured only in the liver may not be reflected in fibroblasts in any way.

About ten or fifteen years ago it was a dogma among biologists that one could not grow these specialized cells in tissue culture. However, it is now known that there is at least one exception to this rule: tumor cells. Tumor cells will grow and divide forever. At the same

time, they will continue to do their specific jobs, at least to some extent. They no longer have a normal complement of chromosomes—somehow their process of mitosis (cell division) has been disrupted, and they end up with irregular numbers of chromosomes. In spite of this, they have a certain usefulness for researchers. The Repository does not stock any human tumor cell lines, but it does provide one line of specialized cells taken from the spleen of mice infected with leukemia virus. Since leukemia is a cancer of the blood, this line allows geneticists to study genes that are responsible for the production of hemoglobin. The cells grow continuously, and after treatment with a chemical (DMSO—dimethyl sulfoxide) they keep on making hemoglobin in culture.

In addition to fibroblasts and tumor cells, two other kinds of human cells can be grown in culture with present methods: lymphoid cells, and cells from amniotic fluid.

The Hazards of Growing Lymphoid Cell Lines

Lymphoid cell lines are grown from lymphocytes, those white blood cells which are part of the immune system. About a decade ago, researchers found that if 1 cubic millimeter of lymphocytes (about 10 million cells) were put into a culture medium and incubated, after a few months some of these cells might start to grow—and that once started, they would be immortal, like tumor cells. But it turned out that inevitably, in such cases, the person from whom the cells were taken was harboring the Epstein-Barr virus, a virus found associated with a rare cancer (Burkitt's lymphoma) as well as with infectious mononucleosis. The next step was the deduction that instead of waiting months for the cells to start growing, one could take anybody's white blood cells, add some E-B virus, and have them growing almost immediately. This proved correct, and the resulting lymphoid lines offer a large number of advantages: They can be grown free in suspension, making it easier to study the transport of different

substances in and out of the cell (Fibroblasts must be attached to a surface and must reach a certain density before they function properly.) Large batches of them can be obtained very rapidly. They reproduce indefinitely. They tend to have a normal number of chromosomes, and they continue to do the specialized jobs of lymphocytes, as well as to express specific genetic defects. However they do represent a possible, though as yet incompletely assessed danger to researchers. The E-B virus may well be a cause of cancer, and even though scientific opinion is divided in this respect—some researchers point out that half the population of the U.S. carries a virus that is indistinguishable from the lymphoma-related virus; some don't believe that E-B virus (a DNA virus) causes lymphoma at all, blaming an RNA virus instead; some claim that only a specific sub-strain of E-B virus is responsible for the tumors—it is clearly a potential bio-hazard. While the Repository carries some 18 lymphoid cell lines taken from patients with cystic fibrosis, galactosemia and other disorders, as well as from normal volunteers, these lines are not distributed without specific guarantees. Any researcher who requests such cells must first agree not to distribute the cells (or their progeny) to other laboratories. Researchers must also agree to abide by a set of minimum safety guidelines developed at NIH in 1974 for working with any lymphoid or virus-transformed lines of human cells.

A Reference Line of Amniotic Cells?

Amniotic cells present a different set of problems and advantages. It would be extremely useful if the Repository could provide reference lines of amniotic cells, giving doctors and researchers the opportunity to check the results of cultures derived from amniocentesis against a known abnormality (or against normal controls.) And in fact, the Repository does have half a dozen cell cultures taken from amniotic fluid, but it keeps them only on a pilot basis because there are still several technical problems. First of all, the cultured

cells may derive from a variety of sources: the fetal bladder, the fetal trachea, fetal skin, or the amnion itself. Even though, with time, the surviving cells appear to be either fibroblasts or more and more like fibroblasts, the quantities of enzymes produced by cells of varying origins may differ. This lack of standardization is a particular problem for those who wish to do biochemical analyses. Secondly, in order to have enough cells to freeze away and distribute, the Repository must grow its amniotic cell lines well past the stage at which they would be grown in amniocentesis (when time is of the essence.) As a result, the two kinds of cells may no longer be comparable. Some of these technical problems may be overcome in the future.

Other types of human cells do not reproduce at all—neither in the body, nor in the tissue culture. Among these are nerve cells, including the brain's neurons, and sex cells—sperm or eggs—which have only half the normal complement of chromosomes because they are waiting to be fused with a mate, to form a complete cell. Why these cells cannot be made to grow and divide is one of nature's mysteries. However, a few researchers have succeeded in growing brain tumor cells that retain some of their original functions in culture.

On balance, then, fibroblasts remain the most attractive human cells for geneticists, especially those who study chromosomes rather than biochemistry.

How To See A *Chromosome*

It is very easy to examine fibroblasts under a microscope while they are being cultured in one of the standard, flat-sided plastic flasks: The entire flask, being transparent, is simply placed on the stage. With a good light microscope, at a magnification of 100, one can clearly see the nucleus of individual cells and tell at a glance whether they have reached the "metaphase" stage of mitosis, during which the doubled chromosomes line up in the center in preparation for the cell's

division into two cells. The only time one can actually look at a chromosome in a living cell is during this stage, which lasts about one hour. At other times, the chromosomes form an indistinguishable mass within the nucleus.

When technicians at the Repository want to prepare cells for karyotyping (a study of their chromosomes), they transfer a few cells to a new flask and cover them with fresh medium, which makes them grow rapidly. On the third day, when a lot of cells are in mitosis, they arrest the mitosis with colchcine. Then they harvest the cells, stain them (which kills them), and prepare slides which can be studied under a microscope. Until 1971, only the general outlines of the chromosomes could be seen, as the stains made them appear solidly of one color. But now, thanks to a new fluorescent dye and the use of trypsin, researchers can distinguish a characteristic pattern of bright and dark bands on each chromosome. Even though molecular biologists still don't understand what causes this banding, it has proved enormously useful to scientists. The most they could do with the old dye was to recognize the signs of Down's syndrome (the extra chromosome No. 21, Trisomy 21, as it is called) and other errors in the total number or gross shape of the chromosomes in the cell. Now that they can recognize individual chromosomes through banding, they are able to diagnose a whole new category of less obvious abnormalities in size or shape.

"The basic structure of chromosomes is not known. The number of strands of DNA in a chromosome, the function of the proteins—these elements are not known. The state of our knowledge of the chromosome is distressingly primitive. There is a lot of work to be done on it which is terribly important, and it is just beginning to get started." **Gordon Tomkins, Chief, Laboratory of Molecular Biology, University of California Medical Center.**

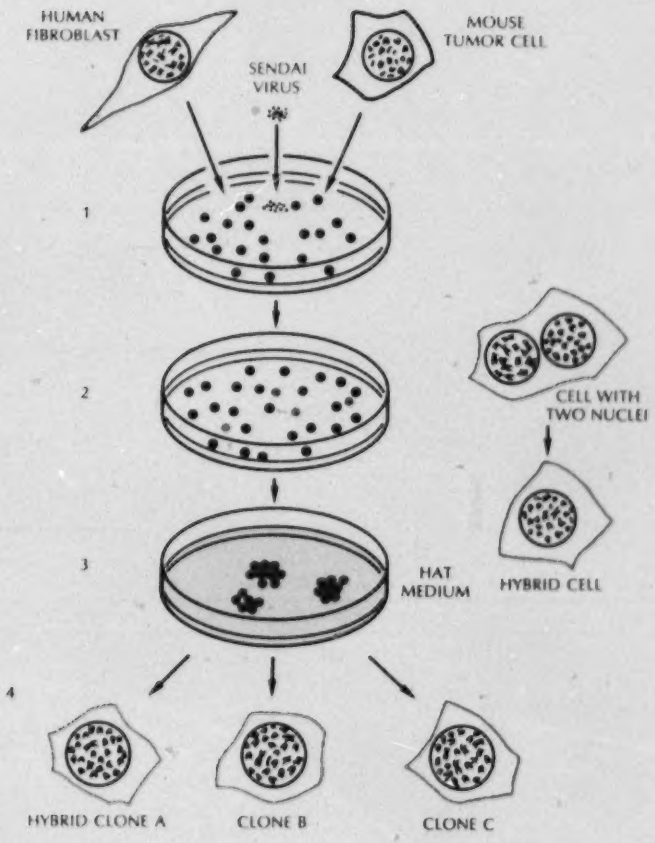
Some people have small pieces of what should normally be on one chromosome erroneously stuck onto the tip of another; geneticists call this a balanced translocation. Since they still have the correct number of genes even though part of one chromosome in pair No. 19, say, is hooked onto pair No. 17, they function perfectly normally. The only problem is that their progeny runs the risk of inheriting only part of this balanced error and ending up with extra or missing genetic material. While most fetuses with such defects are spontaneously aborted, the few that are born may be severely abnormal. It was originally thought that translocations in human chromosomes were rare, but the banding technique has revealed that this is far from true. A large percentage—perhaps 25 percent—of spontaneous abortions have been found to have translocations or other chromosomal abnormalities.

Cytogeneticists are now going through every single line of cells stored at the Repository to study its chromosomes with the banding technique, including cells that were originally sent in for biochemical, rather than chromosomal analysis. The Repository also has 65 cultures that have been well studied precisely because they represent 25 different kinds of translocations, various inversions (in which one piece of a chromosome is turned 180° from its original position) and other abnormalities that show up with the banding technique. These translocations are now proving of particular value to researchers.

The Uses of Man-Mouse Hybrids

Scientific breakthroughs often come about when two technologies are suddenly brought together. This has just happened in human genetics as a result of the new banding technique and an even more revolutionary technique that carries overtones of science fiction: the hybridization of cells from mouse and man.

In mammals, normally only sex cells—egg and sperm, each with a half-complement of chromosomes—can fuse, forming a new and different kind of cell.



Man-mouse hybrid cells are formed by mixing human fibroblasts (skin cells) with mouse tumor cells and adding a fusion-enhancing agent such as inactivated Sendai virus (1). Some of the cells fuse (2), first forming cells with two nuclei and then forming new hybrid cells with a single nucleus. When placed in a special medium, the hybrid cells proliferate and form colonies (3). The hybrid cells retain all the mouse chromosomes but, with continued growth, lose most of their human chromosomes in a random fashion (4). Thus, each hybrid clone (descendant of a single hybrid cell) has a different complement of human chromosomes which can be studied separately.

But as French scientists discovered in the early 1960's, two different lines of non-sex mouse cells, each with a full complement of chromosomes, could sometimes be made to fuse in a culture medium, producing hybrid cells. In 1965, Dr. Henry Harris of the University of Oxford found that such fusion was greatly accelerated by adding a virus to the culture, for the virus formed bridges between adjacent cells. Two years later, researchers at the New York University School of Medicine, Drs. Mary C. Weiss and Howard Green, showed that one could make hybrids between mouse cells and human cells in the same way—and furthermore, that one could recognize the human chromosomes in the hybrid cell.

It remained for a group of scientists led by Dr. Frank H. Ruddle of the Yale University School of Medicine to put the hybrids together with a system of biochemical markers and especially with the new chromosome banding technique, giving birth to a powerful method for the mapping of human genes.

A considerable amount of genetic mapping had been done before this, simply by studying human families. This was particularly easy when the defect was sex-linked, as in hemophilia or color blindness, which are caused by a recessive gene on the X chromosome. (In females, one of the two X chromosomes will usually make up for the other, and there will be no disorder; however, any son born of a woman who carries one defective gene on the X chromosome will stand a 50 percent chance of receiving it—without any compensating gene, since he has no other X chromosome—and of inheriting the defect.) Since human beings could not be used for controlled breeding experiments, however, scientists had to rely on "experiments of nature" in the human population. The use of man-mouse hybrids led to an explosion of knowledge because it overcame this original obstacle to human genetics: Instead of waiting for useful information to be supplied by chance, researchers could now breed human cells at will, using hybridization as a substitute for sexual

breeding. All of a sudden, then, the classical tools of genetics, which had been used so productively on fruit flies and mice, could be brought to bear on the study of human genetics.

This is how it works: The human cells used for hybrids (usually normal human fibroblasts) are brought together with mouse cells derived from tumors—for example, mouse hepatoma (liver cancer) cells. Mixed in a laboratory dish, together with some inactivated Sendai virus, they soon form a few cells with double nuclei, and then new hybrid cells with a single nucleus. Normal mouse cells have 40 chromosomes, and normal human cells have 46, but the hybrids always have fewer than the expected combined total of 86; the usual range is between 41 and 55 chromosomes. A curious trait of these hybrids is that all the mouse chromosomes survive, while most of the human ones are lost. Furthermore, this loss is random—which means that different hybrids will have dfferent sets of human chromosomes.

A New Alphabet of Living Cells

This gives researchers a golden tool: a new alphabet of living cells, each carying only one or a few specific human chromosomes whose activity can be studied independently. And scientists are not lacking in ideas on how to exploit this tool.

Dr. Ruddle has an ambitious plan which should lead to the detailed mapping of all human chromosomes; it begins with the systematic discovery of which genes belong on which chromosome, and will end (some day) with a specific position allocated for each of our thousands of genes. Since he started this work a few years ago, about 100 human genes have been assigned to specific chromosomes for the first time. Dr. Ruddle expects the mapping to grow quite rapidly, for about ten different labs are now working on the problem in the U.S. and abroad, and more are planning to join them. The techniques are also becoming simpler and more efficient.

The new fluorescent dyes make it quite easy to identify each human chromosome by its distinctive banding pattern. Biochemically, each gene can be recognized by the protein it produces, and the proteins produced by the genes of man and mouse can now be separated—even when they appear highly similar—according to the different speeds at which they travel in an electric field. Thus, if two human enzymes are consistently found together in hybrid lines, the corresponding genes must be on the same human chromosome.

The mapping process does not even require 24 different hybrid cell lines, each retaining a single human chromosome (one for each pair of chromosomes and the sex chromosomes.) The Ruddle team recently developed a short-cut: a set of five different hybrid lines, each one selected for its unique subset of eight human chromosomes. The panel of hybrids can then be used

		HUMAN CHROMOSOMES							
		1	2	3	4	5	6	7	8
HYBRID CLONES	A	+	+	+	+	−	−	−	−
	B	+	+	−	−	+	+	−	−
	C	+	−	+	−	+	−	+	−

Panel of three hybrid-cell clones, each containing a unique subset of eight human chromosomes, which can be used for gene mapping. If a specific enzyme is present only in clone C, for instance, its gene must be on chromosome No. 7, the only chromosome with the corresponding pattern of presence (+) or absence (−). A larger panel of five clones, each with a unique subset of the 24 chromosomes, would offer 32 combinatorial possibilities, enough to cover the 24 different human chromosomes.

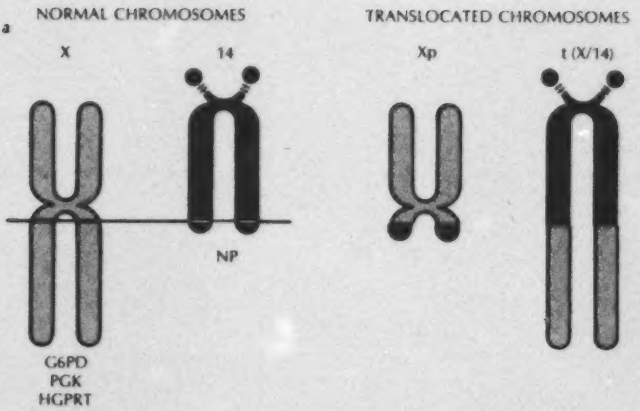
somewhat like a truth table in symbolic logic—to test the sequence in which a particular human enzyme will fit it, and thus reveal to which chromosome the particular gene should be assigned. Five carefully chosen hybrid lines, with 32 combinatorial possibilities, cover all possible pairs of human chromosomes. In practice, however, the team works with 8 to 10 lines, to avoid any false interpretations.

"It's a new syntax of expression," declares Dr. Ruddle. "All you have to do is to examine these eight cell lines, and by a process of elimination it becomes easy to assign any enzyme to its appropriate chromosome." He hopes that eventually the Repository will send out such panels of hybrid cell lines to any researchers who are working on gene mapping or studying specific human enzymes, as this would greatly accelerate the mapping process.

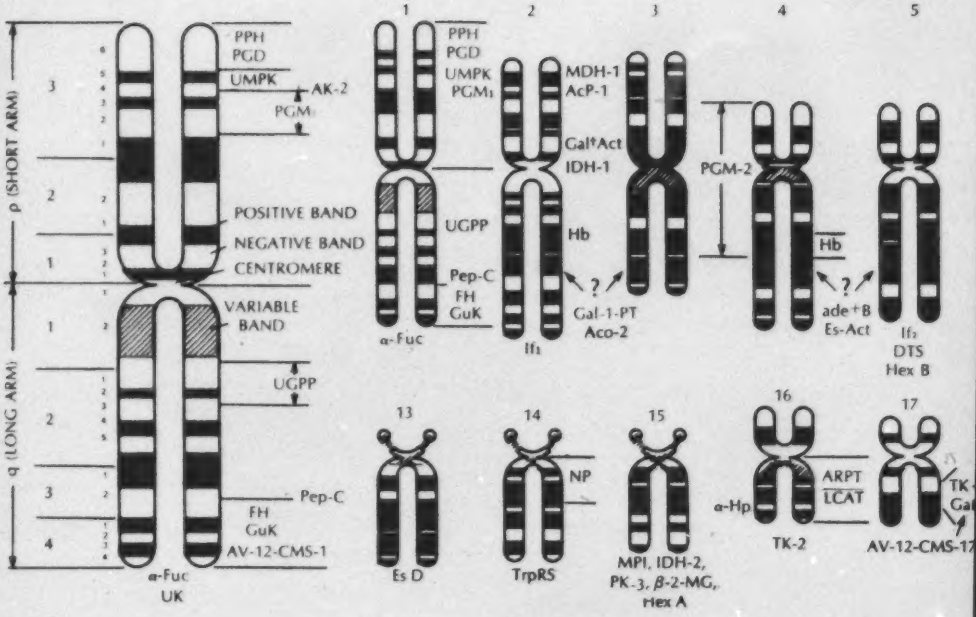
A number of the genes that researchers have already assigned to particular chromosomes are implicated in genetic diseases, he points out, and further knowledge of this kind will increase the ability of doctors to detect such diseases.

Gene Mapping May Revolutionize Medicine

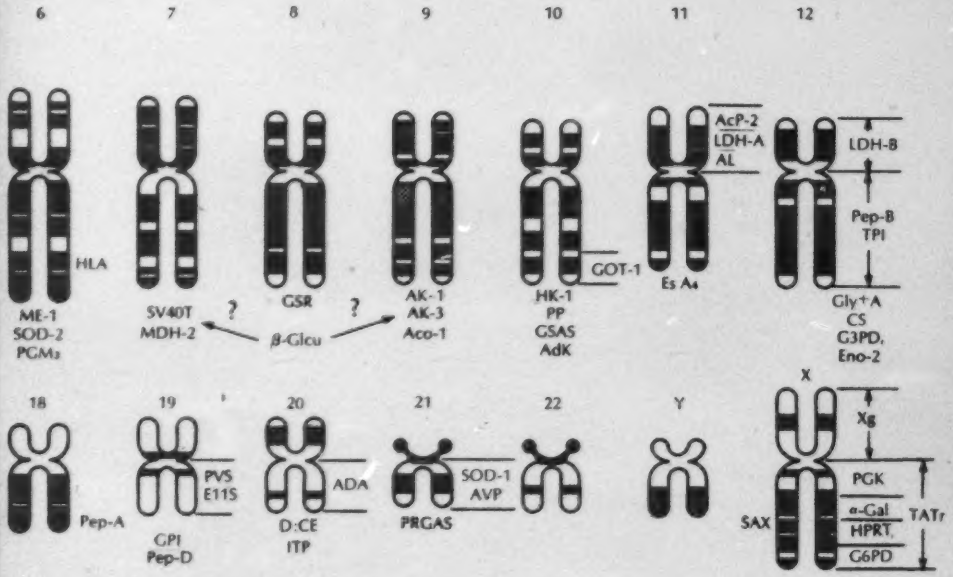
To go beyond this type of mapping and actually locate a gene at a precise point on a chromosome, scientists need additional information. Fortunately, much of it is obtainable from the long list of known chromosomal aberrations. When human cells that are known to carry a specific translocation are hybridized with mouse cells, portions of a chromosome that were originally together may separate. This refines the localization of specific genes. Each chromosomal variant can then be analyzed for the presence of certain enzymes, and it becomes possible to determine, for instance, whether a certain gene is located on the longer part of the translocation—on the chromosome that received extra material—or on the shorter part, which lost some material. The answer is a clue to where the gene would be on a normal chromosome.



Translocations (infrequent genetic accidents in which a piece of one chromosome gets displaced onto another chromosome) help researchers to map the position of a gene within a specific region of a human chromosome. It was known that the three genes which control the production of three specific enzymes (abbreviated as HGPRT, PGK and G6PD) were somewhere on the X-chromosome. To pinpoint the location of the HGPRT gene, scientists capitalized on a translocation which appeared in one family, in which most of the long arm of the X-chromosome had exchanged places with the tip of chromosome No. 14, resulting in two abnormal chromosomes. They fused cells bearing this translocation with mouse cells that were deficient in HGPRT in such a way that only cells bearing the HGPRT gene could survive (this was done by using a special growth medium in which HGPRT-deficient cells would die.) When they analyzed the surviving hybrid clones, they found that each one produced the enzymes PGK and G6PD as well as the mandatory HGPRT. This meant that all three genes must be on a single translocation product. Further analysis led them to the translocated long arm of the X-chromosome. The fact that all three genes were on the long arm was later confirmed by similar experiments with different translocations.



Mapping human genes: Banding patterns and known locations of specific genes are shown for the 22 pairs of non-sex human chromosomes and the two sex chromosomes. Chromosome No. 1 is dawn large (left) to illustrate the standard names and numbers of the various segments. The colored bands represent areas that stain brightly when exposed to a fluorescent dye such as quinacrine; hatched regions are variable bands that stain differently in the chromosomes of different individuals. Genes are designated by the initials of the enzymes or other proteins whose production they specify. Sometimes researchers have established that a gene



is associated with a specific chromosome (for example, α-Fuc, listed under chromosome No. 1.) In other cases they have mapped a more precise location for a gene within a chromosome (for example, GuK, within the long arm of chromosome No. 1.) At times they can do no more than to reduce the possible location of a gene to one of two chromosomes (for example, Gal-1-PT, which may be either on chromosome No. 2 or on No. 3.) All the genes on this map were located through techniques involving hybrid cells. Other genes have been mapped over the years through studies of genetic anomalies in families, but they are not included here.

These advances represent only the tip of the iceberg. The research on mouse-human hybrid cells is leading in many unexpected directions, often of major importance to areas of medicine that seem far removed from genetics. It bears, for instance, on man's resistance to viral infections, and on the whole question of growth and differentiation.

When a virus attacks a human cell, the cell protects itself by making a protein called interferon, which somehow prevents the virus from replicating. This has been known for over a decade, but researchers have been stumped in their efforts to make the body produce more interferon when needed as a defense against virus infections. Since interferon is species-specific—only the kind made by human beings will protect human cells—much could be gained by a better understanding of its mechanism. A fascinating clue has been turned up by members of Dr. Ruddle's team. Working with mouse-human hybrid cells, they discovered that two separate human chromosomes (Nos. 2 and 5) had to be present for the production of human interferon.

Next they learned that simply producing interferon was not enough: the interferon did not do its job of inhibiting virus replication unless yet a third chromosome (No. 21) was also present. This meant that chromosome No. 21 carried a gene for some yet unidentified protein which worked together with interferon to inhibit viral replication in some mysterious fashion—certainly a lead worth following up.

"The double helix brought the realization that information in biological systems could be studied much in the same way as energy and matter. It turned genes into chemical objects whose structure and function could be analyzed and understood in terms of biochemical machinery." **Sydney Brenner, MRC Laboratory of Molecular Biology, Cambridge, England.**

Another exciting development has been the use of hybrid cells to "turn on" specific genes. It is generally believed that each human cell (except sex cells) contains all the genes that are available anywhere in the body. However, only some of these genes are turned on at any particular time in any particular type of cell. Few of the specialized genes are active in fibroblasts, for instance. The genes that produce certain kidney enzymes are turned on only in the specialized milieu of the kidney. The genes for albumin are turned on only in liver cells—not in blood cells. But the Ruddle team has succeeded in making even ordinary human white blood cells produce albumin, when such cells are fused with cells from a mouse liver cancer. All the mouse-human cells that resulted from this fusion produced mouse albumin, and some of them also produced human albumin. This meant that the albumin gene had been right there in the white blood cell all along, but that normally it was repressed or inactivated, and that something in the mouse-liver cell had turned it on.

Learning How To Turn On Specific Genes

Learning how to turn on specific genes in any human cell would open up almost unbelievable possibilities. To begin with, it could make prenatal detection of genetic diseases infinitely more practical. At present, prenatal diagnosis works only when the abnormality happens to be expressed in fibroblasts, the only cells that are easily recovered from amniotic fluid. If fibroblasts— or better yet, fetal blood cells circulating in the mother's bloodstream—could be made to reveal their entire genetic endowment, many more defects could be detected early in pregnancy. Once the "turning-on" and "turning-off" mechanisms were understood, it might also be possible to use it for therapy—to activate certain functions in the fetus, so as to correct specific genetic defects. Even more speculatively, there is a possibility that any group of human cells

could be made to differentiate into a specific organ, such as the kidney, for purposes of transplantation; each person could then have his own properly matched transplant, without waiting for a donor. One could also activate functions that are now found only in neurons, leading to a new era in the understanding of the nervous system and the brain.

That's what the new human genetics is all about: It is laying the groundwork for a new medical science for the 21st century. The discoveries sketched above represent only a fraction of current activity. Other researchers throughout the country are producing equally promising findings in areas too numerous to mention here.

For all of them, the Repository is increasingly important. The new human genetics has very specific requirements, all the more so as it grows in sophistication. It is now clear, for instance, that certain common diseases are not really single entities, but groups of diseases which may be caused by different mechanisms. Diabetes may actually consist of 20 different disorders that only look alike because we don't understand them well enough. The more scientists study such conditions, the more diversified they appear. It is thus essential for researchers to know precisely which subset of a disease they are studying, and the Repository can act as a sort of Bureau of Standards in this respect. For each disorder, it now carries several different lines of human cells clearly labelled as to the age, family history and ethnic origin of the donor, so that laboratory results can be compared more rationally and variations within disease states can be identified. (However, the identity of the donors is kept confidential.)

At the same time, researchers have moved from asking only how an individual gene works to asking how two genes interact. And this may demand very peculiar combinations of cell lines, each of them extremely rare. If these researchers had to fly around the world to get permissions to take samples from critically

ill patients, at great cost, and then spend years trying to grow these cell lines themselves, many of them would give up before they started. The investment in time and money would just be too great. Now they can simply open up the catalogue of the cell bank, order what they need, and get started within two weeks. It is a very real advantage. "It means that competition among scientists need not be at the level of which human cell lines do you own, but at the level of what ideas do you have," explains one geneticist. The Repository is well equipped to find the right cell cultures, take them out of the freezer and revive them overnight, run them through a battery of tests for two or three days to make sure they still do what they are supposed to do, and ship them out in an insulated bag, as a matter of routine.

The new human genetics is seeking answers to some of the most difficult questions now facing medicine. The Repository will vastly improve its chances of finding them, and of saving the lives of an ever-larger number of patients.

"Man is instinctively a mapping animal. He maps the Antarctic Continent; he maps the moon; he maps Mars . . . I would suppose that a map of human chromosomes will be of greater ultimate interest and usefulness to you and me than are the maps of the outer heavens." **DeWitt Stetten, Jr., Deputy Director for Science, National Institutes of Health.**

GLOSSARY

albumin—a protein substance in blood serum.

amino acids—substances from which organisms build protein.

amniocentesis—the withdrawal of a sample of amniotic fluid from the uterus of an expectant mother, by means of a hypodermic needle, in order to test fetal cells for biomedical or chromosomal defects, or fluid for chemical abnormalities.

amniotic fluid—the fluid that cushions the fetus inside the amnion, or sac.

atherosclerosis—a disease of the arteries, in which fatty deposits on the arterial walls obstruct the flow of blood; this may cause heart attacks or strokes.

biopsy—the removal of a piece of tissue from the living body, for examination and diagnosis.

cholesterol—a fatty substance produced in the body and contained in many foods; it is a prominent constituent of the fatty deposits of atherosclerosis.

chromosome—a rod-shaped body, consisting of nucleic acids and proteins and bearing the genes, in the nucleus of a cell.

colchicine—a chemical used to arrest cell division.

culture medium—a nutrient mixture used for growing cells.

cystic fibrosis—a serious disease caused by a recessive gene; it produces thick mucus which prevents normal digestion or breathing.

cytogenetics—the branch of biology that deals with the structural basis of heredity, particularly the chromosomes.

DNA (deoxyribonucleic acid)—the substance of heredity; a large molecule which carries the genetic information necessary for the replication of cells and which directs the building of proteins.

dominant—refers to a characteristic that is apparent even when the gene for it is inherited from only one parent.

Down's syndrome (formerly called mongolism)—a condition involving mental retardation and character-

istic physical features, such as a downward curvature of the eyelids; children born with this condition have an extra No. 21 chromosome.

enzyme—a substance that accelerates (catalyzes) specific chemical transformations.

fibroblast—a connective-tissue cell, found in skin and other tissue.

galactosemia—an inherited deficiency in one of several enzymes that metabolize galactose, a component of milk sugar; one type of galactosemia results in liver damage, cataracts and mental retardation unless it is treated by eliminating milk from the diet soon after birth.

gene—a unit of heredity which is related to a particular characteristic, such as blond hair; genes are segments of the DNA molecule, located in definite positions on specific chromosomes.

genetics—the scientific study of heredity, of how particular qualities or traits are transmitted from parent to offspring.

hemolytic anemia—anemia resulting from an abnormal breakdown of red blood cells.

hemophilia—an hereditary tendency to prolonged bleeding even from slight wounds, due to defects in the clotting factors in the blood; transmitted by a gene on the X-chromosome.

Huntington's disease—a disease that generally appears in adulthood, producing progressive mental and physical deterioration; it is caused by a dominant gene.

hypercholesterolemia—an excess of cholesterol in the blood.

karyotyping—determination of the number and types of the chromosomes in cells.

lipoproteins—compounds of fatty substances and protein; the lipoproteins in blood carry cholesterol.

lymphocytes—white blood cells which are part of the immune system.

lymphoma—cancer of lymphoid tissue.

metabolism—the processes by which cells assimilate food for growth, or break it down for energy.

mitosis—cell division.

mycoplasma—tiny organisms, smaller than bacteria but larger than viruses.

otolaryngologist—medical specialist in diseases of the ear and larynx.

Petri dish—a small, shallow dish of thin glass, with a loose cover, used for cultures.

PKU (phenylketonuria)—an inability to properly metabolize the amino acid phenylalanine, leading to nerve and brain damage and retardation; it can be diagnosed from abnormalities in the urine, and its effects can be minimized by a diet that excludes phenylalanine.

recessive—refers to a characteristic that is apparent only when genes for it are inherited from both parents.

receptor sites—specific areas on a cell membrane which sense the environment in which the cell lives.

tissue culture—the growth of cells in nutrient solutions that duplicate their normal environment.

translocation—the transfer of a fragment of one chromosome onto another chromosome.

trypsin—a pancreatic enzyme used to separate clumps of cells.

X-chromosome—the sex chromosome which occurs singly in male cells and in duplicate in female cells.

Y-chromosome—the male sex chromosome (normal males carry one X-chromosome and one Y-chromosome.)

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